membranes of confluent monolayers. However, recent studies suggest that in epithelial cells, the expression of CFTR at the apical membrane depends on the level of cellular differentiation and the acquisition of cell polarity.

Colonic cells that have not formed polarized monolayers fail to target CFTR to the plasma membrane. These unpolarized cells retain CFTR in a perinuclear location and show no activation of plasma membrane anion conductance in response to cAMP. Iodide efflux assays show that cAMP enhances anion efflux from polarized HT29 cells, but fails to activate either the parental unpolarized cell line or the polarizing cells prior to confluence. After polarization, these cells show apical membrane CFTR staining and cAMP-activated Cl secretion, detected as a transepithelial Cl current. Expression studies show that both the polarized and unpolarized HT29 cells express equivalent amounts of CFTR mRNA and protein. In addition, CFTR is expressed in the non-polarized cells as the fully processed glycosylated protein so that in these cells, CFTR has encountered the Golgi glycoprocessing enzymes. Yet, CFTR is retained in a post-Golgi intracellular compartment, perhaps in vesicles of the trans-Golgi network, until the cells form tight junctions and discrete apical and basolateral membrane domains. This apical targeting mechanism is shared by some other apically-directed glycoproteins, such as proteases and hydrolases and differs from that observed for the Na/K-ATPase, which is targeted to and retained at the basolateral membranes. The signals responsible for intracellular CFTR retention in non-polarized cells are as yet unknown. Nevertheless, these studies demonstrate that the expression of this component of the Cl secretory machinery depends on cellular differentiation and may, therefore, be similar to the intermediate stage of epithelial differentiation thought to occur in polycystic kidney disease. The CFTR retention signals characteristic of non-polarized epithelial cells are apparently not expressed in non-epithelial cells, which target CFTR to the plasma membrane. It will be interesting to determine whether these epithelial targeting signals influence the cellular location of mutant CFTRs.

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Maturation of solute transport mechanisms in pulmonary epithelia

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Recent studies have indicated that the epithelium lining the distal (alveolar) regions of the fetal and perinatal lung do not merely represent a passive barrier to the movement of fluid and solutes, but rather actively transports ions. *In utero*, the fetal lung is normally filled and distended with fluid which results from the active secretion of Cl (~5 ml/kg/hr of fluid) by the epithelium. The presence of this fetal lung liquid is critical for the normal development of the lung. At birth, the lung must become air filled and become a net fluid absorbing organ. During labor there is a rapid switch from fluid (Cl) secretion to fluid (Na) absorption which arises, at least in part, from the increase in circulating endogenous hormones with β_2 agonist properties. The active Na transport by the distal lung epithelium plays a critical role in the clearance of lung liquid from the newborn's air spaces; impaired Na transport resulted in respiratory distress, hypoxemia, and elevated lung water contents in otherwise healthy newborn animals. Na transport is equally important in the postnatal lung, as it plays a critical role in the clearance of pulmonary edema. Recent studies have shown that the distal lung regions of the human lung are similar to the lungs of lower animals and absorb fluid by active Na transport.

As in other Na transporting tissues, there is a Na/K-ATPase located on the interstitial (basolateral) side of the epithelial monolayer which creates an electrochemical gradient for Na movement across the cell's membrane and an apical (alveolar side) entry pathway for Na. Although some Na absorbing epithelia may utilize Na exchangers or symports, these plasma membrane proteins do not play a role in fluid absorption from the perinatal lung's air spaces. Rather, Na transport by both the perinatal and adult alveolar epithelium utilizes Na channels.

It is now known that there are a family of epithelial Na channels that have no structural, biochemical, or nucleotide similarity to the voltage-regulated Na channel found in excitable cells. Since polyclonal antibodies which recognize the putative amiloride-binding subunit of the renal epithelial high-amiloride affinity Na channel label fetal and adult alveolar epithelial apical membranes and recognize polypeptides in alveolar epithelial membrane vesicles, it is likely that alveolar epithelia have at least one type of of Na channel which is antigenically similar to the renal epithelial Na channel. There is biochemical and physiologic evidence for more than one type of Na channel in rat alveolar epithelium. Patch clamp studies have identified a 25 pS nonselective cation channel (NSC), along with 12 pS and 4 pS highly selective Na channels in the apical membrane of the alveolar epithelium. These Na permeant ion channels are all amiloride sensitive. Membrane vesicle, Ussing chamber and whole cell patch clamp studies also indicate that there are both H-type and L-type (EIPA) sensitive Na channels on the perinatal alveolar epithelium's plasma membranes. In addition, there are two populations of independent ^{3}H-benzamil binding sites in membrane vesicles made from perinatal alveolar epithelium. Whether these different alveolar epithelial Na channels are in different alveolar epithelial cell subtypes, and whether they arise from the same or different genes is unknown.

A recent major breakthrough has been made using functional expression cloning techniques to determine the primary structures of the putative pore forming subunit of the rat epithelial Na channel (αrENaC) and two highly homologous subunits (β and γrENaC) of the rat epithelial Na channel. The latter two subunits markedly increase the Na channel activity when their cRNA is co-expressed with αrENaC cRNA in *Xenopus laevis* oocytes. All three subunits have only two transmembrane spanning regions

and likely are part of novel cation channel family which is highly conserved from an evolutionary point of view; it shares homology with relevant proteins from C. *elegans* to humans. It is likely that αrENaC codes for the pore-forming subunit of one of the amiloride sensitive epithelial Na channels; however, until lipid bilayer reconstitution experiments are carried out one cannot rule out that the αrENaC codes for a regulatory protein that is intimately related to the Na channel. Studies have, however, demonstrated that similarly sized αrENaC transcripts are present in appropriate Na transporting tissues but are absent in tissues such as the liver and brain. Expression of αrENaC is developmentally regulated in the rat and human with the timing of the expression correlating with the lung's ability to transport Na. Expression of arENaC is increased by glucocorticosteroid hormones, which correlates with these hormone's ability to accelerate the maturation of Na transport in the intact developing lung.

The signal transduction pathways regulating Na transport have been extensively investigated in the kidney, and adult and perinatal alveolar epithelium. All three epithelia increase Na transport when intracellular [cAMP] increases; however the responsible cell membrane receptor is different. Although kidney epithelia can respond to AVP, V_2 membrane receptors are absent on perinatal alveolar epithelia, thus explaining why AVP does not increase alveolar epithelial Na transport. Similarly, the alveolar epithelium's Isc is unresponsive to aldosterone stimulation, and membrane permeant analogues of cGMP or PKC agonists do not increase its Na transport. In contrast, the alveolar epithelium increases [cAMP] and Na transport following β_2 receptor stimulation. The mechanism of action is complex and includes a β_2 agonist induced increase in intracellular [Ca], a nearly 2 log shift in the sensitivity of the NSC sensitivity to Ca, and a reduction in intracellular [Cl] which directly increases the open probability of the 25 pS amiloride-sensitive NSC in perinatal epithelium.

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Chloride transport inhibitors in epithelia

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Epithelial Cl transport involves the synchronized operation of Cl uptake systems, such as the Na/2Cl/K-cotransporter, and a Cl exit step, usually Cl channels. Cl transport can be inhibited at both sides of the epithelium. Loop diuretics such as furosemide, bumetanide and torasemide inhibit binding to the Na/2Cl/K-cotransporter by their high infinity. Cl channel blockers inhibit the Cl exit step. This mechanism of inhibition has been shown to be very effective in the thick ascending limb of the loop of Henle (TAL). However, the same compounds (NPPB) which inhibit Cl absorption in the TAL at <1 μmol/liter, have much less effect on the secretion of Cl in the colon. The reason for the different affinities of these blockers in various epithelia may be based on the differences of the respective Cl channels. Currently much evidence favors the view that the intermediate conductance outwardly rectifying Cl channel (ICOR) is not the key Cl exit mechanism in epithelia such as colonic crypt cells. Rather, it appears likely the small- or very-small-conductance Cl channels are responsible for epithelial Cl secretion. Unlike the ICOR-channel, these small channels are not very sensitive towards NPPB and related compounds.

In a broad search for putative inhibitors of Cl secretion we have also examined cromanol with the internal number 293 B. This compound proved to be a very potent inhibitor of Cl secretion in the colon. 293 B is a racemate. A separation of the two enantiomers 407 B and 434 B revealed that the latter compound is biologically active with an IC50 of only 250 nmol/liter from the serosal side.

In Ussing chamber experiments we examined whether 293 B is specific for cAMP-mediated Cl secretion. 293 B inhibited the equivalent short circuit current produced by forskolin, 8-CPT-cAMP, IBMX, VIP, PGE_2, adenosine, and cholera toxin. It was ineffective on the secretory Cl current produced by the Ca-ionophore, ionomycin. To examine the effect of 293 B in more detail, individual colonic crypts were perfused *in vitro* and the voltage across the basolateral membrane (V_{bl}) was measured. It was shown that agonists acting via cAMP induce a rapid but transient depolarization followed by a sustained, less pronounced depolarization. During this phase the membrane conductance of the impaled cell was maximal. 293 B added to the basolateral side depolarized V_{bl} further by 6 to 10 mV. This suggests that 293 B reduced a K conductance. However, 293 B had no effect on the basal K conductance, nor on the K conductance induced by ATP. Comparable whole cell patch clamp studies in CFPAC-1 cells, which show little or no Cl current in response to cAMP, have revealed that 293 B also inhibited a cAMP-induced K conductance. The present data indicate that Cl transport can be inhibited reversibly by a new class of inhibitors of the cromanol type (293 B). These substances apparently inhibit a K conductance which is activated by cAMP. Inhibition of this conductance then limits the amount of Cl secreted.

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